S/N 10/500,435 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	KAMATA, et al.	Examiner:	C. Babic
Serial No.:	10/500,435	Group Art Unit:	1637
Filed:	June 28, 2004	Docket:	10873.1416USWO
Title:	METHOD OF EFFECTING LYSIS OF ACID-FAST BACTERIA AND METHOD OF PERFORMING GENE AMPLIFICATION DETECTION THEREWITH		

CERTIFICATE UNDER 37 CFR 1.6(d): I hereby certify that this paper is being transmitted by facsimile to the U.S. Patent and Trademark Office on August 2, 2006.
By: [signature]
Name: GINA M. DAHL

DECLARATION UNDER 37 CFR 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Yuji Izumizawa, declare as follows.

I graduated from Okayama University Graduate School of Pharmaceutical Science in March 2001, where I majored in pharmaceutical chemistry and conducted a study on mutations of bacteriophages induced by ultraviolet light. I joined ARKRAY, Inc. in April 2001 and was assigned to Research and Development Department. From then on, I have been engaged in the research on a genetic test system for infectious diseases.

I am a named inventor of the application identified above. I had the following experiments conducted to show the significance of the use of particular non-ionic surfactants in the lysing of acid-fast bacteria.

Procedures

1) Preparation of Samples Containing Acid-Fast Bacteria (BCG)

BCG was cultured in a liquid culture medium (trade name: MycoBroth, Kyokuto Pharmaceutical Industrial Co., Ltd.) for one week. Then, the liquid culture medium containing the cultured BCG was diluted with a physiological saline solution until a turbidity corresponding to #1 of the McFarland turbidity standard was obtained. Thereafter, the resultant diluent was further diluted with a physiological saline solution so as to attain predetermined dilution factors ((1) 5×10^3, (2) 10^4, (3) 5×10^4, (4) 10^5, (5) 5×10^5). Thus, test solutions containing the BCG were prepared. 100 µl of each of the test solutions was centrifuged at 12,000 g for 10 minutes, and the supernatant was then removed. Thus, the BCG in the form of pellet was obtained. The pellets obtained from the respective test solutions were used as samples to be subjected to a lysis treatment.

2) Lyses Treatment

Three types of lysis reagents shown below were provided. Each of the following reagents was dissolved in a TE Buffer (10 mM EDTA and 25 mM Tris-HCl, pH 8.0).

	Non-ionic detergent
1% TE-Triton (Example)	1% polyoxyethyleneglycol p-t-octylphenyl ethers (Triton)
1% TE-Tween 20 (Comparative Example 1)	1% polyoxyethyleneglycol sorbitan alkyl esters (Tween 20)
2% TE-Tween 20 (Comparative Example 2)	2% polyoxyethyleneglycol sorbitan alkyl esters (Tween 20)

100 µl of each lysis reagent was added to the samples, and the resultant mixtures were heated at 96°C for 10 minutes so as to lyse the BCG.

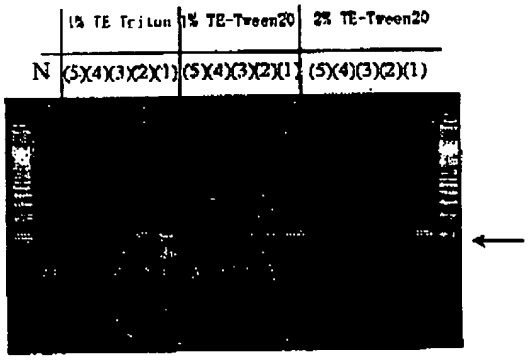
3) PCR Reaction

In order to confirm whether the BCG was lysed, PCR was performed for the samples that had been subjected to the lysis treatment, and the PCR amplification reaction products obtained were subjected electrophoresis. The PCR and

electrophoresis were carried out in the same manner as in Example 2-1 of the present application (WO 2003/097816). More specifically, the PCR was carried out in the following manner. Primer sequences used and the composition of a PCR reaction solution were the same as those in Example 2-1. 2 µl of each of the samples that had been subjected to the lysis treatment was added to 23 µl of the PCR reaction solution, and the PCR amplification was carried out with respect to the resultant mixture.

Result of Experiment

The following is a photograph showing the result of the electrophoresis. The numbers (1) to (5) shown above the photograph correspond to the above-described dilution factors, respectively.



Comparing the bands at the respective lanes (3) (dilution factor: 5×10^4), it was found that the band detected was strong in the case of the TE-Triton, whereas the band detected was weak in the case of the 1% TE-Tween 20 and the band was not detectable in the case of the 2% TE-Tween 20.

Moreover, comparing the bands at the respective lanes (1) (dilution factor: 5×10^3), it was found that the band detected in the case of the TE-Triton was stronger than those detected in the cases of the 1% TE-Tween 20 and 2% TE-Tween 20.

Although the above experiment was conducted using BCG as acid-fast bacteria, the result shown would be expected to be seen with other acid-fast bacteria, because acid-fast bacteria basically are common in that "they have a high cell-wall lipid content".

From the result of the above experiment, it can be said that Triton can achieve a better lysing effect than Tween 20.

I further declare under the penalty of the law of perjury of the laws of the United States that the foregoing is true and correct to the best of my information and belief.

Respectfully submitted,

Dated: August 1, 2006

By: _____
Yuji Izumizawa

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